What makes a chemical a respiratory sensitizer?

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Purpose of review

In this article we consider the characteristics that are associated with chemical respiratory allergens, and that may be essential for effective sensitization of the respiratory tract.

Recent findings

Chemical respiratory allergens share some characteristics with other chemical allergens, specifically chemical allergens that cause skin sensitization and allergic contact dermatitis. The unique and defining characteristic of chemical respiratory allergens, which in most instances distinguishes them from contact allergens, is the ability to provoke the preferential development of T helper 2-type immune responses. There are, in addition, other characteristics, such as the ability to increase matrix metalloproteinase expression or to cause perturbation of redox homeostasis, that may in some instances facilitate the induction or expression of respiratory allergy, but it is not yet clear if these attributes are common or essential properties of all chemical respiratory sensitizers.

Summary

Predicting which chemical allergens may selectively induce respiratory sensitization is an important objective, but remains a significant challenge because our understanding of the relevant physicochemical characteristics and biological properties that confer on chemicals respiratory allergenic potential is incomplete.

Keywords

chemical respiratory allergens, cytokines, skin sensitization, T helper 2 cells

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Abbreviations

MMP	matrix metalloproteinase
TDI	toluene diisocyanate
Th	T helper

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Introduction

The title of this article implies that there may exist certain characteristics that are common to chemicals known to cause allergic sensitization of the respiratory tract. Our purpose is to explore that possibility. However, as a prelude to that, it is necessary, and helpful, to provide some working definitions, and to identify some confounding factors.

For the purposes of this commentary, allergy is defined as the adverse health effects that may be provoked following the stimulation of a specific immune response. The corollary is that by definition we are considering here chemicals that are able to provoke an immune response such that allergic sensitization of the respiratory tract will be acquired. However, it is necessary to appreciate that not all chemical allergens that are able to provoke a specific immune response will have the potential to cause sensitization of the respiratory tract. The majority by far of chemical allergens are associated with skin sensitization and allergic contact dermatitis [1], and are believed not to effect sensitization of the respiratory tract. Other chemical allergens, far fewer in number (and the subject of this article), are associated instead with respiratory allergy and occupational asthma and are rarely, if ever, implicated as causes of skin sensitization [2]. Why different chemical allergens are associated with different forms of sensitization and occupational illness is a fascinating issue, and one to which we will return. However, with regard to these introductory remarks it is important to emphasize that many important characteristics of chemical allergens are common to both contact and respiratory sensitizers.

Finally, there remains a debate about whether different classes of chemical respiratory allergens share the same mechanism of sensitization. Although, as emphasized above, such allergens, by definition, provoke immune responses, it is possible that the immunological effector mechanisms through which sensitization is achieved vary.

Rationale for considering the characteristics of chemical allergens *per se*

It is relevant first to consider the characteristics of chemical allergens *per se*. The justification for adopting this approach is twofold. First, as indicated above, it is clear that contact allergens and chemical allergens associated with sensitization of the respiratory tract do have some important features in common. Second, there is reason to suppose that chemical respiratory allergens can induce sensitization of the respiratory tract when experienced by

routes other than inhalation, and of particular relevance from an occupational health perspective is that dermal exposure may be effective in this respect [3]. Related to this is an interesting observation that represents something of a paradox. Experience suggests that chemical respiratory allergens will elicit positive responses in experimental animal models designed specifically to identify skin sensitization potential. This is despite the fact that many such respiratory sensitizers are rarely associated with contact allergy [4]. Thus, for example, phthalic anhydride, a known human respiratory allergen, has never been implicated definitively as a skin sensitizer, despite opportunities for occupational dermal exposure [5]. Nevertheless phthalic anhydride elicits positive responses in guinea pig and mouse skin sensitization assays [6,7]. The reason why the apparent intrinsic potential (based on data from animal models) of chemical respiratory sensitizers to induce contact allergy fails to translate into a dermal health risk in an occupational setting is unclear. However, these data do suggest that chemical respiratory allergens may represent a special subset of chemical allergens generally.

Common properties of contact allergens and respiratory allergens

The characteristics of chemical allergens have been considered previously elsewhere, primarily within the context of skin sensitization [8••]. One critical requirement that is common to all chemical allergens is the need to form a complex with protein. In their native state chemicals are not immunogenic; that is they are unable to provoke an immune response. The term hapten is often used to describe a chemical that usually has a single antigenic determinant, but which requires stable association with a carrier molecule to stimulate a response. The need for covalent interaction between chemical allergens and proteins is articulated in what has become known as the electrophilic theory of sensitization [9]. However, it is important to appreciate that, in practice, inherent electrophilicity does not necessarily confer on a chemical sensitizing potential. Neither does the lack of inherent electrophilicity always prohibit sensitization. Metabolic activity in the skin may result in the conversion of what have been described as 'prohaptens' into protein-reactive derivatives [10]. The conclusion drawn is that chemical allergens are inherently protein reactive, or must be converted at the relevant tissue site into a protein-reactive species. Nevertheless, it remains a possibility (as yet unproven) that discrete classes of chemical allergens (contact and respiratory sensitizers) display qualitative differences with respect to association with protein at the amino acid level.

A second requirement is that the chemical is able to gain access to the appropriate tissue compartment. The assumption is that for both contact and respiratory chemical allergens the target tissue is epithelium – in the case of contact allergens the epidermis and for respiratory allergens either the epidermis or respiratory epithelium. Access to the viable epidermis requires successful passage across the *stratum corneum*, while no such barrier guards the epithelium of the respiratory tract.

One approach to comparing and contrasting the necessary properties of contact and respiratory allergens is to review the requirements for skin sensitization for which greater consideration has previously been given. It is known that for the successful induction of skin sensitization a contact allergen must have the following characteristics to clear various biological hurdles: effective skin sensitizers must gain access to the viable epidermis; they must be inherently protein reactive, or metabolized to a protein-reactive species; they must cause sufficient local trauma in the skin to stimulate the production/increased production of those cutaneous chemokines and proinflammatory cytokines that are known to be required for the activation of local dendritic cells, those resident in the epidermis being Langerhans' cells (Langerhans' cells are responsible for transporting antigen from the skin to regional lymph nodes, and for its subsequent presentation to responsive T lymphocytes); and they must be inherently immunogenic and capable of provoking an immune response of the magnitude sufficient for the development of sensitization [8••,11]. It can be concluded also that the degree to which sensitization is acquired (that is the extent of priming) is directly proportional to the vigour of the induced immune response, and, in the case of contact allergens, the magnitude of the clonal expansion of allergen-reactive T lymphocytes [12]. By comparison, it can be assumed that chemical respiratory allergens also have a requirement to gain access to viable epithelium (in the relevant target tissue), that they must form stable associations with protein to acquire immunogenicity, and that they must engage and activate local dendritic cells in order that allergen is transported to regional nodes and presented effectively to T lymphocytes. It must be assumed also that chemical respiratory allergens will be inherently immunogenic and that the magnitude of induced immune responses will govern the level of sensitization acquired.

Although collectively these analyses allow definition of some properties that a chemical respiratory allergen must possess, they clearly do not provide the whole picture, since left unanswered is the question of why some chemicals preferentially elicit skin sensitization whereas others are associated largely, or exclusively, with sensitization of the respiratory tract.

What differentiates chemical respiratory allergens from contact allergens

Against the background of generic properties that are associated with chemical allergens *per se*, the questions

become what distinguishes chemical respiratory allergens from contact allergens, and what unique characteristics do the former have? It is now possible to address the first of these questions, but the answer to the second remains elusive. That is, the evidence indicates that chemical respiratory allergens differ from contact allergens with respect to the quality of immune response they elicit preferentially [13–15,16•]. However, the physicochemical properties of chemical allergens that dictate the type of sensitization they will cause remain largely uncharted.

The fundamental difference at an operational level is that chemical respiratory allergens appear to stimulate the development of selective type 2 responses (the quality of immune response that favors – *inter alia* – sensitization of the respiratory tract). In contrast, contact allergens in the main appear to induce preferential type 1 immune responses that, almost by definition, favour skin sensitization.

Much of the evidence for the elicitation by contact and respiratory chemical allergens of discrete qualities of immune response (T helper (Th)1 and Th2-type selective responses, respectively) derives from extensive studies in rodents [12–15,16•,17–20,21•], although there is reason to suppose that the same or similar differences may also hold true in humans [22,23]. The assumption from studies in rodents is that chemical respiratory allergens provoke the development of polarized immune responses that result in the development of Th2-type T lymphocytes and that the cytokine products of these cells facilitate the production of allergen-specific IgE antibody, and support immediate-type allergic hypersensitivity responses [24–31]. The difficulty in translating these observations directly to humans is that there remains some uncertainty about a universal mandatory role for IgE antibody in the pathogenesis of occupational respiratory allergy to chemicals. Although it is clear that confirmed chemical respiratory allergens can and do induce IgE antibody responses in exposed individuals, a number of investigations have found that a proportion of symptomatic individuals (and in the case of diisocyanates, sometimes a substantial proportion of those with symptoms) lack demonstrable IgE antibody [32–35]. This apparent paradox has been explored in detail previously [36,37]. In brief, it is likely that the correlation between IgE antibody and occupational respiratory allergy is considerably closer than is sometimes assumed. This conclusion is based upon the fact that, for various reasons, the presence of IgE antibody may be missed: because inappropriate analytical methods have been used, because analyses have been conducted too long after the last exposure, or because the levels of IgE antibody in target tissues are not reflected by those in plasma (for further details and considerations, see [32,36,37]). However, it must be acknowledged that, even if the association in humans between respiratory allergy to chemicals and IgE is somewhat stronger than has sometimes been reported, there may exist mechanisms of allergic sensitization that are independent of IgE antibody; and this may be true especially of diisocyanates. However, even if IgE-independent mechanisms do have an important or decisive role to play in some circumstances, the available evidence indicates that the successful development of occupational chemical respiratory allergy is nevertheless associated with selective Th2-type immune responses [22,23].

It can be concluded, therefore, that in the main, chemical respiratory allergens are characterized by an ability to elicit preferential type 2 immune responses that favour (via IgE-dependent or independent mechanisms) the acquisition of sensitization of the respiratory tract and the subsequent elicitation of respiratory allergic reactions. The question remains, however, why it is that this subset of chemical allergens displays this immune selectivity. Although attempts have been made to discern structure–activity relationships that may describe chemical respiratory allergens [38], the number of confirmed allergens in this class is comparatively small and it would be fair to conclude that there has as yet been no clear definition of the physicochemical characteristics that confer on chemical allergens this immune selectivity.

At a biological level there has in recent years been a growing interest in the possibility that qualitatively divergent immune responses are orchestrated by dendritic cells. Broadly, the theory is that dendritic cells of different phenotypic sub-populations, dendritic cells at different stages of differentiation/maturation, or dendritic cells resident within different tissue microenvironments cause the initiation of discrete (type 1 or type 2-selective) immune responses, and there is some evidence to support this [39–42]. Although little is known of the influence of dendritic cell populations on the development of qualitatively divergent immune responses to different forms of chemical allergens, we have in this laboratory recently made some observations that may prove relevant. Investigations in mice have suggested that contact and respiratory chemical allergens may cause the mobilization of tissue-resident dendritic cells with different kinetics, and that this may in turn impact on the phenotype and functional characteristics of dendritic cells migrating to, and arriving in, regional lymph nodes, and, as a consequence the class of immune response that will preferentially be triggered (Cumberbatch, Clelland, Dearman, Kimber, unpublished data). These investigations continue.

Other relevant features of chemical respiratory allergens

If the defining characteristic of chemical respiratory allergens is that they induce a qualitatively polarized class of

immune response, it is nevertheless relevant to examine whether there exist other features or functions that may facilitate the acquisition of pulmonary sensitization.

One interesting observation has been an association with matrix metalloproteinases (MMPs), a family of proteolytic enzymes that have as their substrates extracellular matrix proteins. In a mouse model of asthma to toluene diisocyanate (TDI) a variety of pathophysiological markers was found to be reduced significantly following administration of MMP inhibitors [43], probably resulting from downregulation of the accumulation of inflammatory cells, and via abrogation of induced proinflammatory cytokine expression [44•]. Of particular interest is the fact that there is evidence for the increased expression of MMP-9 in the sputum of patients with TDI asthma following challenge with the sensitizer [45••]. The hypothesis is that TDI directly results in the overexpression of MMPs, including MMP-9, with a resultant increase in airway inflammation and remodelling that facilitates the development and persistence of allergic and asthmatic symptoms. The question is whether this facility to cause an upregulation of MMPs (and/or a downregulation of their natural inhibitors, the tissue inhibitors of metalloproteinases) is a characteristic of other allergenic diisocyanates, or of other classes of chemical respiratory sensitizers.

There are intriguing indications for the existence of occupational asthma susceptibility genes. For certain chemicals, associations have been reported with genes that map within the major histocompatibility complex (MHC) on chromosome 6, with increased frequencies of some class II antigens (DQA1*0104 and DQB1*0503), but not class I antigens, observed in individuals with TDI asthma [46,47]. Other class II alleles (HLA-DQ5 and HLA-DR1) have been demonstrated to be overexpressed in patients with occupational asthma to acid anhydrides [48•,49••]. That there may exist associations between respiratory allergy to certain chemicals and genes within the MHC is not totally unexpected, but what is potentially more informative (at least in the context of this article) are apparent associations with genes coding for glutathione S-transferases (GSTs) [50–52]. The evidence indicates that there exists an apparent association between certain GST loci and occupational asthma to diisocyanates. Such correlations are of interest when considered within the context of studies in animals that have suggested that inhalation exposure to diisocyanates causes airway epithelium barrier dysfunction that is, in part, dependent upon glutathione levels [53–55]. Moreover, independent investigations have indicated that diisocyanates may disrupt thio-reductase homeostasis in the airways associated with a reduction in glutathione levels [56,57]. As there is no doubt that thiol-redox balance is closely linked to inflammatory processes, it may prove that disruption of airway epithelial cells by diisocyanates, and resultant changes in redox status, may facilitate allergic sensitization and allergic disease. Again, it is not clear whether such associations extend beyond diisocyanates, and whether the ability to disrupt redox balance is a prerequisite for occupational respiratory allergy and asthma on exposure to low molecular weight compounds, or a common characteristic of chemical respiratory allergens.

Conclusion

Chemical respiratory allergens share some characteristics with other chemical allergens, specifically chemical allergens that cause skin sensitization and allergic contact dermatitis. Among these are the ability to form stable and immunogenic associations with proteins, the ability to reach epithelial tissues and engage with dendritic cells, and the ability to induce the production of cytokines and chemokines required to drive the immunobiological processes that together facilitate the initiation of an immune response. The unique and defining characteristic of chemical respiratory allergens, that in most instances distinguishes them from contact allergens, is the ability to provoke the preferential development of Th2-type immune responses. There are, in addition, other characteristics, such as the ability to increase MMP expression or to cause perturbation of redox homeostasis, that may in some instances facilitate the induction or expression of respiratory allergy, but it is not yet clear if these attributes are common or essential properties of all chemical respiratory sensitizers.

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